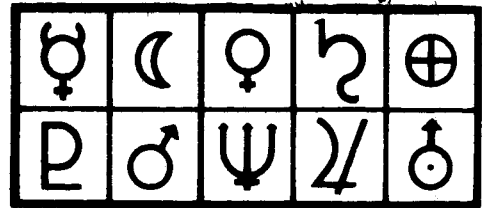


QR 8

MARCH 1968



PLANETARY QUARANTINE

SANDIA LABORATORIES QUARTERLY REPORT - PLANETARY QUARANTINE PROGRAM

PLANETARY QUARANTINE DEPARTMENT 2570

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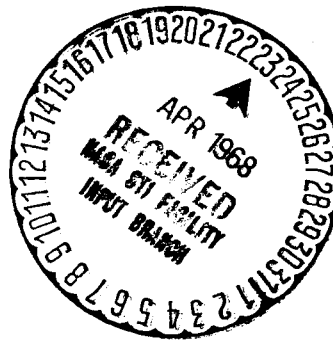
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Sandia Laboratories Quarterly Report - Planetary Quarantine Program

Eighth Quarterly Report of Progress

for

Period Ending March 31, 1968

Planetary Quarantine Department
Sandia Laboratory, Albuquerque, New Mexico

March 1968

Project Nos. 340.229.00
340.229.01

This work was conducted under NASA Contract Numbers R-09-019-040 and H-13245A.

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The following is a summary of the activities being pursued, and the progress made, during the first quarter of calendar year 1968.

1. Objective Hierarchy

- A. Description. In order to determine what actions are needed to achieve planetary quarantine objectives on a cost-optimal basis, an "objective hierarchy" is being constructed. A description of this approach is given in "A Systems Approach to Contamination Control", appearing in the Proceedings of the NASA/AEC sponsored Symposium on Current and Advanced Concepts in Instrumentation and Automation in Contamination Control (see Publications).
- B. Progress. During this quarter, an objective hierarchy was developed in a non-quantitative manner through fourteen objective levels. The analysis begins with international planetary quarantine objectives and works "down" to such items as: gross factors affecting bioburden at launch, gross factors affecting bioburden release, gross factors affecting bioburden change in space, and so forth. A report on this work should be available in the coming quarter.

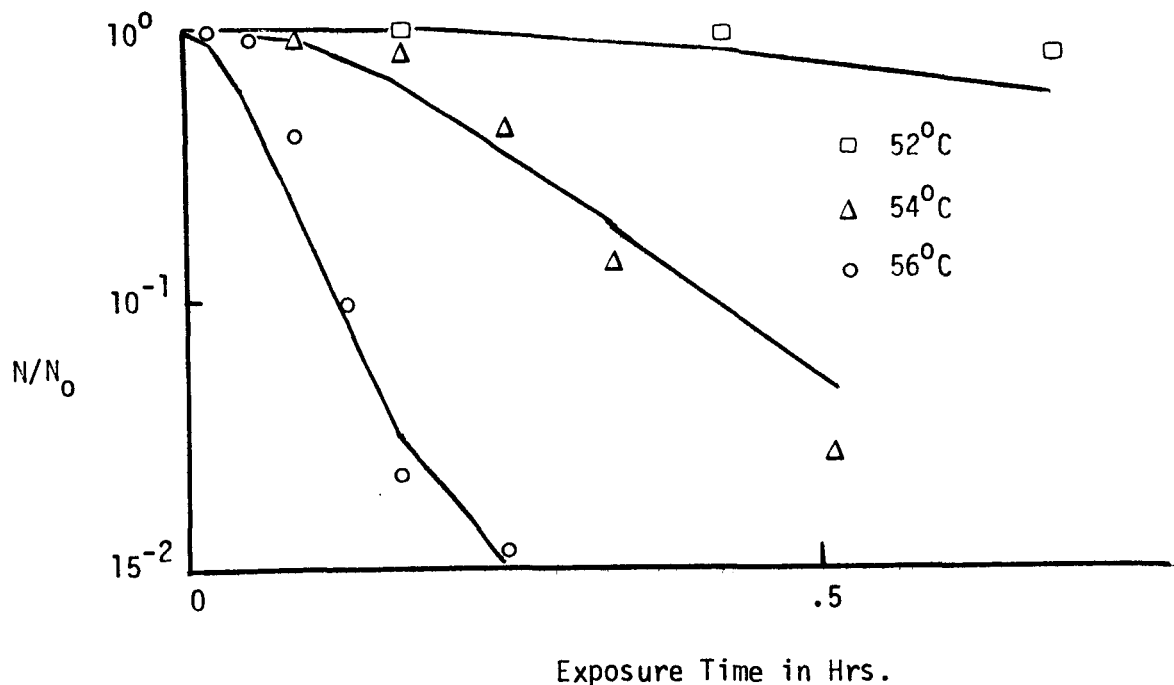
2. Microbial Death Models

- A. Description. A reliable model of microbial deactivation is needed for reasons described in quarterly report, QR 5, and in "A Rational Model for Spacecraft Sterilization Requirements", Sandia Laboratories Research Report, SC-RR-67-256.
- B. Progress. This quarter, tests of model flexibility were carried out for data for thermal sterilization of yeast. The data was generated at 52°C, 54°C and 56°C by Woods. [T. H. Woods, "Lethal Effects of High and Low Temperature on Unicellular Organisms",

Adv. Jn. Biol. and Med. Physics, 4, p. 119-165 (1956)].

Since the model was derived on a rational basis, it was assumed that it should also approximate the situation encountered in yeast sterilization as well as that encountered with bacterial spores (previously reported on). The yeast data was selected for "flexibility" tests since the curves at 52°C and 54°C are convex while the 56°C curve is sigmoid.

Results are shown in the accompanying figure. Although the "fits" are not optimum, the illustration does demonstrate compatibility. This represents a fit of the data at three temperature levels in such a fashion that the chemical reaction rate constants appearing in the model are related to one another by the Arrhenius equation at these different temperatures. The analysis



was completely computerized, and constraints suggested by bacterial cell structure were not removed for these yeast tests.

Also, some progress has been made this quarter in determining the manner in which water activation affects survivor curves based on the way it affects the entropy and enthalpy of certain chemical reactions in proteins.

3. Implementation System

A. Description. The objective of this activity is to, ultimately, design a system, based upon our problem analysis, that will provide the outputs necessary for meeting planetary quarantine responsibilities.

B. Progress. Progress this quarter has been made in system design for meeting both lunar and planetary responsibilities.

A report analyzing long-term requirements and current status is complete. This addresses system design in a broad way for meeting planetary responsibilities, and presents a formal analysis of such a system for meeting lunar responsibilities.

Additionally, in the area of lunar responsibilities, implementation system activities during this quarter have centered on the analysis and design of a computer software system for accepting, storing, processing and outputting spacecraft contamination information. This work is a continuation of that started in the aforementioned report. The personnel in the Public Health Service Sterility Control Laboratory at Cape Kennedy are cooperating in this detailed design effort.

The Planetary Quarantine Information System is considered to consist of three secondary parts:

- (i) personnel for providing data to the system, operating the system, and making meaningful uses of system outputs;
- (ii) a computer system capable of appropriate data storage, calculations and outputs, and;
- (iii) software capable of directing the activities of the computer system to provide efficient data handling, processing and to make suitable outputs available at the right places and times.

The personnel and computer system aspects of the implementation system are addressed in the report mentioned above. The software aspect is now being addressed in detail. Our objective is to provide complete programming specifications, with documentation, for an implementation system for lunar responsibilities.

In analyzing software needs, two categories of software were considered. First, computer codes related to the models used in predicting spacecraft and lunar bioburdens, and second, system computer code for internal data handling, application of model codes to the data, and communication among various parts of the implementation system.

Models developed here are being coded as they are completed. The major design effort this quarter has been in analyzing and designing the system computer code.

To date, we have determined: many of the data requirements; methods for data identification, storage and updating; system outputs; and methods for outputting information.

All of the data requirements will not be known until a complete set of models exists. System outputs have been tentatively

determined pending NASA approval. A "dialogue" has been designed for communicating with the computer system to retrieve these outputs. This takes the form of questions from the computer with answers from the Planetary Quarantine Officer. This "inter-change", depending upon the sequence of answers given, will permit the Planetary Quarantine Officer to retrieve hundreds of pieces of information.

The system is being designed in such a way that data for lunar decisions and certifications, along with the predictions based on those data, will be stored on magnetic discs for easy access by the Washington-located console. Additionally, all information will be stored on tape to permit whatever additional recall that might become necessary. An efficient retrieval system has been designed.

Information available as outputs to the console will fall into four categories for the lunar system: (i) Apollo; (ii) Unmanned lunar missions, (iii) Environmental information and (iv) Lunar inventory.

The "Apollo" category has been designed to provide information regarding the identification of astronauts, suits and spacecraft modules assigned to each Apollo flight and to provide quantitative and qualitative predictions of bioburdens on each module and complete spacecraft as of the last assay date, the launch date, and the lunar impact date. This information can be retrieved from the system in a variety of ways. Some of the modeling for this is described below.

The "Unmanned" category will relate bioburdens of the now completed program of unmanned flights to current lunar burdens and subsequent probabilities of lunar sample contamination from these sources. This will be done with the lunar retrieval model discussed briefly later.

The "Environmental" category, as designed, will provide information regarding the quantitative and qualitative microbial environments being experienced in the various Cape facilities used for spacecraft preparation. It will also provide listings of spacecraft modules in each facility.

The "Lunar Inventory" category will provide information concerning the predicted amounts and locations of microbial contamination on the lunar surface. It will also relate that inventory to probabilities of contaminating lunar samples.

The basic analysis undertaken for design purposes is in the form of trees. Flow charts have been and are being developed to help provide the specific programming guidance for the system.

4. Estimating and Predicting Surface Bioburdens

A. Description. In sampling spacecraft surfaces for purposes of predicting the microbial burden, one encounters several problems.

First, there is the problem of estimating the microbial burden at a given point in time based upon direct surface samples taken at that point in time.

Second, there is the problem of predicting the microbial burden at future times based upon prior direct surface sampling and upon whatever environmental samples that are available.

Third, and common to both the first two problems, there is the problem of the degree of confidence that one may place in such estimates and predictions.

It seems hopeful that the first of these problems can be resolved in a manner which will prove satisfactory in the Apollo program.

The solution of the second problem depends, to a large extent, upon the solution to the first problem. Additionally, one must be able to correlate environmental measurements with a spacecraft bioburden.

What follows is a survey of the work being done by Sandia Laboratories in these areas this quarter. The discussion centers primarily around problems one and two.

- B. Progress. In approaching the problem of microbial burdens on surfaces, one is confronted with a curious situation. In any fixed environment there seems to be an upper limit upon the number of microorganisms that one finds on a unit area of surface independent of time. This apparent phenomenon has been termed the "plateau". Thus, any model used to estimate or predict microbial burdens should account for such observations. Incidentally, the "plateau" is observed even when spores are known to be present. Thus, we begin with some simple assumptions.

In order to explain the "plateau", it is assumed that the great majority of microorganisms are deposited upon surfaces attached to larger ambient particles (dust, and so forth). Such an assumption appears necessary since (i) it takes much higher energies to remove naked spores from surfaces than one normally

finds exerted on spacecraft surfaces and (ii) if spores are not removed, one would not expect a "plateau" in reasonable periods of time since spore "death" seems unlikely in any reasonable time period based on their demonstrated "longevity".

The assumption that microorganisms, in particular spores, adhere to larger environmental particles appears to be consistent with physical theory. This assumption allows one to conclude that mechanical removal of microorganisms from surfaces is not only possible, but likely, under normal working conditions.

Under the conditions envisioned above then, as time passes, microorganisms may be deposited on a spacecraft surface, and such organisms as deposited may be removed by mechanical means or they may die. Thus, the problem becomes a "birth (deposition) and death (mechanical removal or 'death')" process. Mathematical descriptions of such processes can be found in elementary probability texts. Thus, only a rudimentary description will be given here.

We let

$P_n(t)$ be the probability that there are exactly n viable particles on the surface at time t .

Here, a viable "particle" means one that is attached to, or is itself, a viable microorganism.

It is assumed that the particle deposition rate, $d(t)$, is independent of the number of particles present on the surface at time t . This would seem to be a perfectly reasonable assumption so long as the number of particles is not extremely large.

It is further assumed that the removal rate $R_n(t)$ is proportional to the number of particles present, and that the proportionality factor does not depend upon n , i.e.,

$$R_n(t) = r(t) \cdot n, \text{ for all } n.$$

This is tantamount to assuming that, independent of the number of particles present, each particle present at time t has the same probability of being removed. In general, this seems reasonable for fixed sizes of particles. For example, when "particles" means microorganisms and "removal" means death, this situation obtains when one is using almost any model of death in which the microorganisms die independently of one another and each has the same probability of death at time t .

If, in addition, the particle deposition rate is bounded above, i.e.,

$$d(t) \leq d,$$

and the removal "factor", $r(t)$, is bounded away from zero.

$$r(t) = r > 0,$$

then the mean,

$$M(t) = \sum_{n=0}^{\infty} n P_n(t)$$

of the standard birth and death process is bounded above,

$$M(t) \leq \frac{d}{r}.$$

But $d(t)$ is surely bounded above, and, having assumed that ambient particles carry the microorganisms, it is reasonable that $r(t) \geq r > 0$ even for spores. Thus, the conditions are no more restrictive than the earlier assumptions, and on the basis

of those, the above bound on the mean number of particles present obtains. On average then, the birth and death model accounts for the plateau.

Having exhibited a plateau, one is justified in inquiring about steady state solutions to the birth and death equations. It is easily demonstrated that the steady state solutions \hat{P}_n satisfy

$$P_n = \frac{1}{n!} \left(\frac{d(t)}{r(t)} \right)^n \exp \left[- \left(\frac{d(t)}{r(t)} \right) \right].$$

From this it can be deduced that

$$\hat{P}_n = \frac{1}{n!} \left(\frac{d_{AV}}{r_{AV}} \right)^n \exp \left[- \left(\frac{d_{AV}}{r_{AV}} \right) \right]$$

where d_{AV} and r_{AV} are time averages of $d(t)$ and $r(t)$, respectively, after a steady state has been reached. In particular, for the steady state mean, \hat{M} ,

$$\hat{M} = \frac{d_{AV}}{r_{AV}}.$$

Experiments to gain some confidence in this model were performed this quarter. They were designed to check the steady state mean predicted by the model.

To check the steady state form of the birth and death model, an experiment using vegetative cells (Staph. aureus) as the particles in question was performed. These organisms were dispersed from a nebulizer and allowed to settle-out onto plastic petri dishes. One cloud of microorganisms was dispersed every 30 minutes, with samples and controls taken every 30 minutes. Twenty-five clouds were dispersed.

Based on a rough plot of the data so obtained, it appeared that a plateau existed for data taken in clouds 18 through 25, that is in the 9 to 12 1/2 hour time period. During this period the mean number of organisms per petri dish, as assayed, was 713. This is, then, the experimental mean of the plateau. The average fallout rate during this same period was experimentally determined to be 102 particles per petri dish per 30 minute period.

Independently, the removal factor was found to be 15% per 30 minute period for Staph. aureus on dry petri dishes in the same environment in which the fallout portion of the experiment was performed. This represents "death", and it was assumed that no mechanical removal took place since the particles were collected in petri dishes. The birth and death model predicts a steady state mean

$$\hat{M} = \frac{d_{AV}}{r_{AV}} = \frac{102}{0.15} = 680 \text{ organisms per petri dish,}$$

comparing quite favorably with the 713 organisms per petri dish that was observed. Similar experiments were performed with essentially the same result.

Based on these experiments, one has some confidence that the birth and death model can be used "locally". But since the derivation of the steady state birth and death model is valid only for surfaces each part of which see the same environment, it is unlikely that it is applicable to the entire spacecraft. A theoretical extension has been completed, but again, this is based on some physical assumptions. Experimentation has been initiated to check the reasonableness of these assumptions.

5. Vacuum Probe Development

- A. Description. The vacuum probe was developed to remove surface contamination for evaluation of microbial content. The probe has been used at Sandia for laboratory studies and is being further developed for field use.
- B. Progress. Since Dr. Martin S. Favero of USPHS proposed and is showing that sonication of the filter, cone, and tip of the vacuum probe raises the assay efficiency, the probe cone and tip were redesigned to make them shorter. Making the cone and tip shorter allows the parts to be insonated in much less liquid, which is an advantage in plating out the microbes.

This design was decided on as the final design for evaluation. Additional vacuum probes are currently being constructed for evaluation by the USPHS at Phoenix and at Cape Kennedy and for use here in laboratory experiments. The parts of the probe are being cast from an aluminum-magnesium alloy by the foundry at Sandia.

Several innovations for ease in use have been incorporated into the design. The cone is much easier to remove than on the earlier models. A bevel prevents the "O" ring from falling out. In addition, the device is very light and easily handled.

Rudy Puleo and Richard Graves of USPHS at Cape Kennedy recently visited and discussed some of the practical aspects of using the probe to actually monitor Apollo for microbial loading.

An attachment for the probe is also being developed to allow the probe to be used outside clean room areas. A

filtered air sheath immediately surrounding the probe tip is the basic technique being developed.

6. Fine Particle Studies

- A. Description. Experimentation conducted during this quarter was oriented toward behavior of random particles on surfaces. In order to conduct such studies realistically, random particles (size, shape, density, etc.) should be used to simulate actual conditions expected during spacecraft assembly conditions which occur in uncontrolled areas as well as in clean rooms. This laboratory, and others, have studied natural occurring particles for behavior on surfaces with limited success. Most of such studies have been based on bacterial content of particles for indicators; however, several problems with random or ambient particles make such studies very difficult. The main problem is that of low bacteria content on random particles, and the difficulty of obtaining control or reference bacterial levels. Part of this experimentation has been directed toward developing random test particles that can be detected repeatably.
- B. Progress. Four methods are currently being studied to provide particles that simulate ambient or random particles.
1. Tag ambient particles with an indicator. The technique under study is that of electrostatically charging naturally occurring particles which are combined with oppositely charged spores. A laboratory device has been completed and is undergoing tests to tag particles in this way. Preliminary tests show promise for production of tagged ambient particles.

2. Spinning Top Aerosol Generator. One method of producing particles that seemed applicable to this problem was the spinning top aerosol generator. The apparatus consists of a top shaped rotor which is suspended on an air bearing and driven with air pressure. Liquid introduced on the center of the upper portion of the rotor quickly spreads over the top of the rotor and is thrown off the edge as very uniformly sized droplets.

Two alternative procedures could be used here. The spores could be suspended in a solution of a chemical that leaves residue when aerosolized as a droplet and allowed to dry. This method would leave spores entrapped in a particle composed of the chemical used. Alternatively, concentrated suspensions of spores only could be used. By producing fairly large droplets, clusters of spores would be formed after the droplets dried. With careful control, particles of the sizes and varieties of consistencies needed in this research could be produced.

Presently, an apparatus of this type has been borrowed from Lovelace Foundation and modified for this experiment.

3. Large, viable particle cultivation. As a possible alternate to the dust particle tagging technique, various sized fungal spores are being considered in the fallout studies. At the present time, we have two workable spore suspensions. One is a Mucor spp. measuring 4μ is diameter and the other is a Helmenthosporium spp. which is football shaped measuring $10 \times 25 \mu$.

A new method for harvesting fungal spores of this nature has been developed and yields spores that are relatively free of the associated vegetative cells or mycelia. The method involves a surface active agent in the rinse water which wets the spores which are still attached to the mycelia. Once wetted, the spores are separated from the mycelia by insonation, and then the rinse water containing the spores is decanted. Subsequently, the spores are washed to remove excess nutrients and vegetative cell fragments. These spores are stored in deionized water in the refrigerator as opposed to refrigerated 95% ethanol for Bacillus subtilis var. niger spores.

4. Spore and Dust Compost. One last method considered, but incompletely examined so far, is suspending spores in water, mixing them with a water suspension of dust, and allowing the mixture to dry. The resulting cakes could be crushed and sized for use in these experiments. This approach will be pursued in the near future. Equipment is on hand for this study.

7. Contamination Control Study (NASA Contract No. H-13245A)

A. Description. This project is the preparation of a contamination control handbook containing technical information in the field of contamination control. It will provide an authentic source of technical information and data useful to persons engaged in the field of contamination control.

B. Progress. The significant levels of accomplishment during the quarter are shown in the following activities:

1. Approximately 150 pages of new handbook material was prepared in rough draft form bringing the total to about 550 pages. This material includes technical information, tables, charts and sketches on the following subjects:
 - a. An introduction to the handbook and the field of contamination control.
 - b. Contamination control and the interrelation of the many factors involved in a contamination control program.
 - c. Verification of surface cleanliness giving types and methods of testing including limitations and problems involved.
 - d. Contaminants in gases and gas systems with information on specifications and analysis of gases, effective handling, control and use.
 - e. Contaminants in liquids and hydraulic systems and methods of detecting contaminants in liquids.
 - f. Air supply conditioning and monitoring of air-borne contamination.
 - g. An introduction to microbial contamination and the monitoring of microbial contaminants in air, gases, liquids, solids and surfaces.
 - h. Handling and storage with techniques for contamination controls during in-process handling, and storage and transport.
2. Bibliographies for 7 of the 10 sections requiring them were completed and included in the page count of new material for this quarter.

3. The following subjects were reviewed and revised for preparation of final draft:
 - a. Contamination control (Sect. 1)
 - b. Contamination control in product design (Sect. 2)
 - c. Microbial contamination (Sect. 6)
 - d. Handling and storage (Sect. 9)
4. Research, acquisition and preparation of material was initiated or continued on the following subjects:
 - a. Contamination control in gases and liquids.
 - b. Removal of contaminants from liquids.
 - c. Radiation.
 - d. Personnel.
 - e. Test and maintenance of airborne contamination control facilities.
 - f. Glossary
5. Arrangements were made with the Sandia Technical Information Department to provide technical editorial assistance in preparing the material in final form for publication.
6. Messrs. D. M. Garst and/or K. F. Lindell visited the following activities to obtain information and material for the handbook:
 - Western Electric Company, Allentown, Pennsylvania
 - Air Products and Chemical Co., Specialty Gas Div., Emmaus, Pennsylvania
 - Baker Chemical Co., Specialty Gas Dept., Phillipsburg, New Jersey
 - National Bureau of Standards, Gaithersburg, Maryland
 - Turco Products, Wilmington, California

7. Mr. Quintin T. Ussery from the Manned Spacecraft Center, Houston, Texas, visited 2572 to discuss material being incorporated into the handbook.

8. Publications

- a. J. P. Brannen and R. E. Haymond, Alabama Research Institute, "Non-Iterative Solutions to Functional Equations", was accepted for publication by COMPUTING.
- b. C. A. Trauth, Jr., "A Multi-Stage Decision Model for Mission Non-Contamination Requirements", SC-DC-67-2051, has been accepted for publication in the Journal of Space Life Sciences, Dordrecht, Holland.
- c. C. A. Trauth, Jr., "A Systems Approach to Contamination Control", SC-M-68-94, presented to and published in proceedings of AEC/NASA Symposium on Contamination Control, Albuquerque, New Mexico, Sept. 12-14, 1968.

9. Presentations

- a. Presentations on the space program were made by H. D. Sivinski to the following groups during this quarter:
 - 1. Physics classes at Rio Grande High School on February 20, 1968, entitled, "Man in the Space Environment".
 - 2. Technical Lecture Series on Contamination Control for Industry presented by the West Coast Chapter of the American Association of Contamination Control, University of Southern California, Los Angeles, California, February 28, 1968, "An Examination of Government and Technical Society Documents".
 - 3. The American Society of Mechanical Engineers student section at The University of Colorado, Boulder, Colorado, January 10, 1968, entitled, "Man in the Space Environment".
 - 4. U.S. Department of Health, Education, and Welfare, National Clinic on Technical Education, Western Skies, Albuquerque, New Mexico, March 28, 1968, entitled, "Man in the Space Environment".

- b. W. J. Whitfield, "The Basic Function of a Clean Room", presented to the ASTM Seminar, February 6, 1968, in Chicago, Illinois.
- c. W. J. Whitfield, participated in a meeting to revise the "NASA Standard Procedures for the Microbiological Examination of Space Hardware", in Phoenix, Arizona, January 11, 1968.
- d. J. P. Brannen, "An Application of Mathematical Biology to Spacecraft Sterilization", University of Alabama Research Institute, Huntsville, Alabama, March 26, 1968.
- e. H. D. Sivinski, presented lecture and was panel chairman at the Rio Grande Chapter of the American Association for Contamination Control Symposium (on Contamination Control in Research), held at the Western Skies Hotel, Albuquerque, New Mexico, March 1, 1968.

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